mechanism is observed, in which the turn is always found to be the major determinant in initiating the folding process, followed by cooperative formation of the inter-strand hydrogen bonds and the side chain packing. Furthermore, direct transition to the folded state from fully unstructured conformations does not take place. Instead, the native hairpin is always observed to form from partially structured conformations involving a non-native (ESYI) turn from which the native (NPDG) turn forms, triggering the folding to the beta-hairpin.

1039-Plat

Residue Specific Analysis of Frustration in Folding Landscape of Repeat Alpha/Beta Protein Apoflavodoxin

Antonios Samiotakis[1], Loren Stagg[2], Dirar Homouz[3], Margaret S. Cheung[1], Pernilla Wittung-Stafshede[4].
[1]University of Houston, Houston, TX, USA, [2]Rice University, Houston, TX, USA, [3]University of Texas - Medical Branch, Galveston, TX, USA, [4]Umea University, Umea, Sweden.

Topological frustrated proteins can give rise to complex folding pathways and transition state profiles. To better understand such systems, we combine experimental and computational methods to study *Desulfovibrio desulfuricans* apoflavodoxin by producing several point mutation variants. By equilibrium unfolding experiments, we first revealed how different secondary-structure elements contribute to overall protein resistance towards heat and urea. Next using stopped-flow mixing coupled to far-UV circular dichroism (CD), we probed how individual residues affect the amount of structure formed in the experimentally-detected burst-phase intermediate. Together with *in silico* folding route analysis of the same point-mutated variants and computation of the growth in nucleation size during early folding, computer simulations suggested the presence of two competing folding nuclei at opposite sides of the central β-strand 3 (i.e. at β-strands 1 and 4), which cause early topological frustration (*i.e.,* misfolding) in the folding landscape. Particularly, the extent of heterogeneity in the folding nuclei-growth correlates with the *in vitro* burst phase CD amplitude. In addition, Φ-value analysis (*in vitro* and *in silico*) of the overall folding barrier to apoflavodoxin's native state revealed that native-like interactions in most of the β-strands must form in transition state. Our study reveals that an imbalanced competition between the two sides of apoflavodoxin's central β-sheet directs initial misfolding while proper alignment on both sides of β-strand 3 is necessary for productive folding.

1040-Plat

Folding Kinetics of IkB: Excursions Through the Energy Landscape

Ryan M.B. Hoffman, Patricio O. Craig, Ingrid L. DeVries, Elizabeth A. Komives, Peter G. Wolynes.
University of California, San Diego, CA, USA.

The IkB proteins, inhibitors of the transcription factor NF-kB, are comprised of tandem repeats of a conserved primary structure. The tandem repeats are stabilized as a repeated tertiary structural motif, at least when complexed with NF-kB and DNA. Structural fluctuations in the native state deviate substantially from a simple repeating pattern, as reported by site-resolved hydrogen exchange rates, NMR chemical shifts and relaxation parameters, and energetically frustrated intraprotein contacts.
Here we have studied the folding of the first four ankyrin repeats of human IkB-alpha and -beta, using coarse-grained structural models to extensively simulate dynamics under structurally-parameterized Hamiltonians. The folding reaction coordinate was sampled with biasing potentials and the free energy as a function of the reaction coordinate was calculated with weighted histogram analysis. The trajectories were used to assign the thermodynamic changes between substates in the folding mechanism. Kinetic models connecting the folding substates were used to predict experimentally known (un)folding rates.

1041-Plat

Protein Folding Landscapes for Alpha- and Beta-Miniproteins Using All-Atom Simulations with an Optimized Force-Field

Jeetain Mittal[1], Robert B. Best[2].
[1]Lehigh University, Bethlehem, PA, USA, [2]University of Cambridge, Cambridge, United Kingdom.

With recent advances in simulation methodology and computer hardware, it has become possible to access physical phenomena occurring on the microsecond timescale with molecular dynamics simulations. Even though most naturally occurring proteins fold on a much slower timescale (milliseconds to seconds), the design of miniproteins (e.g., trpcage, trpzip, beta-hairpin) and proteins (e.g., Villin) which fold close to the protein folding-speed limit (~ 1 μs) have made feasible direct, high resolution, simulations of protein folding. At the same time, the shortcomings in the current energy functions for proteins are becoming increasingly evident with clear biases in secondary structure preference of different force fields being reflected in which proteins they are able to fold. To be able to compare and complement experimental observations, an ideal force-field would be able to fold different types of secondary structure without additional modifications or inputs.
We will present results from extensive replica exchange molecular dynamics simulations for folding of GB1 beta-hairpin and trpcage (representing beta and alpha structures respectively) with a force field based on AMBER ff03 and optimized only to reproduce the helix-coil transition. We obtain converged equilibrium distributions for runs starting from a completely unfolded state and from the native state, with folded populations at room temperature in quantitative agreement with experiment. The folded structures for both the proteins (starting from a completely unfolded structure) have average backbone dRMS from the experimental structures of less than 1 Å. Finally, we will present results for the 35-residue protein Villin. Although convergence of equilibrium distributions in this case is not computationally feasible, we nonetheless obtain a folded structure within 1 Å dRMS of the native structure, starting from a completely unfolded coil-like conformation.

1042-Plat

Amyloid Fiber Precursors in Native and Denaturing MD Simulations of an IG Light Chain Domain

Nina Pastor[1], Cesar Millan-Pacheco[1], Francisco Murphy-Perez[1], Daniel Alejandro Fernandez-Velasco[2].
[1]Universidad Autonoma del Estado de Morelos, Cuernavaca, Morelos, Mexico, [2]Universidad Nacional Autonoma de Mexico, Mexico, DF, Mexico.

The native structure of proteins is generally necessary for function. Mutations and changes in the environment can lead to altered conformations that are prone to aggregation, leading to the formation of amorphous or fibrillar aggregates, which are associated with diseases such as systemic amyloidoses. Precursors for these aggregates can come from any point in the folding route for the protein, and there is a direct correlation between poor protein stability and its potential to form fibers. Immunoglobulin variable light chain domains are prominent in primary systemic amyloidoses; in particular, class 6a appears in ~40% of clinical cases, despite being expressed only in ~2% of B-cells under normal conditions. 6aJL2 is a prototype for these protein domains, containing the germline sequence of the VIa gene fused to the lambda J2 segment, built to explore the properties of this particular class. An allotypic variant, containing the R24G mutation, is even more susceptible to fibril formation, and is also less stable. Both forms are two-state folders. Starting from the crystal structure of 6aJL2, we carry out MD simulations of the original 6aJL2 protein and the R24G variant under native conditions (298K) and under strong denaturing conditions (573K), to explore the native basin and the unfolding pathway of these proteins, in order to identify possible precursors for the formation of fibers. We compare the results of the simulations to available circular dichroism, fluorescence and NMR data. Preliminary data provide a rationale for the lower stability of R24G, due to the loss of both stacking and H-bond interactions. Analysis of both native and denaturing simulations suggest that unfolding for both variants starts at a proline-rich loop and makes the N-terminus of the protein available for seeding the amyloid.